CLAIMS

What is claimed is:

A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:

- a) annealing a polynucleotide of interest to free oligonucleotide primers having known sequences of N nucleotides in length to generate annealed primers;
- b) subjecting the annealed primers to a single base extension reaction to extend the annealed primers by the addition of a terminating nucleotide;
- c) observing the identity of each terminating nucleotide that has been added to the annealed primers.
- 2. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:
 - a) annealing a polynucleotide of interest to oligonucleotide primers having known sequences of N nucleotides in length under hybridization conditions, to generate annealed primers;
 - subjecting the annealed primers to a single base extension reaction which comprises providing to the annealed primers nucleotides corresponding to each of the four bases, to extend the annealed primers by the addition of a terminating nucleotide;
 - c) observing the identity and location of each terminating nucleotide that has been added to the anyteried primers.
- 3. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:
 - a) attaching an array of oligonucleotide primers having known sequences of N nucleotides in length to a solid support at known locations;
 - b) annealing the polynucleotide of interest to the array of oligonucleotide primers to generate annealed primers;
 - c) subjecting the annealed primers to a single base extension reaction to extend the annealed primers by the addition of a terminating nucleotide;

- d) observing the identity and location of each terminating nucleotide within the array on the solid support.
- 4. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:
 - a) attaching an array of oligonucleotide primers having known sequences of N nucleotides in length to a solid support at known locations;
 - b) annealing the polynucleotide of interest to the array of oligonucleotide primers to generate annealed primers;
 - c) subjecting the annealed primers to a single base extension reaction to extend the annealed primers by the addition of a terminating nucleotide;
 - d) selecting a starting annealed primer;
 - e) observing the identity and location of the terminating nucleotide which has been added to the starting annealed primer, to determine the next nucleotide in sequence;
 - f) selecting a second annualed primer which has the same nucleotide sequence as nucleotides 2 through N of the starting annualed primer nucleotide plus the next nucleotide in sequence as determined in step (e), and
 - g) repeating steps (e) and (f), using the second annealed primer as the starting annealed primer for each repetition, to determine the sequence of the polynucleotide of interest.
 - 5. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:
 - a) attaching an array of oligonucleotide primers, having known sequences of N nucleotides in length to a solid support at defined locations;
 - b) annealing the polynucleotide of interest to the array of oligonucleotide primers under hybridization conditions, to generate annealed primers;
 - subjecting the annealed primers to a single base extension reaction which comprises providing to the annealed primers nucleotides corresponding to each of the four bases, to extend the annealed primers by the addition of a terminating nucleotide;
 - d) observing the identity and location of each terminating nucleotide within the array on the solid support.

- 6. A method of analyzing the sequence of a polynucleotide of interest, comprising the steps of:
 - a) attaching an array of oligonucleotide primers, having known sequences of Nnucleotides in length to a solid support at defined locations;
 - b) annealing the polynucleotide of interest to the array of oligonucleotide primers under hybridization conditions, to generate annealed primers;
 - subjecting the annealed primers to a single base extension reaction which comprises providing to the annealed primers nucleotides corresponding to each of the four bases, to extend the annealed primers by the addition of a terminating nucleotide;
 - d) selecting a starting annealed primer;
 - e) observing the identity and location of the terminating nucleotide which has been added to the starting annealed primer, to determine the next nucleotide in sequence;
 - selecting a second annealed primer which has the same nucleotide sequence as nucleotides 2 through N of the starting annealed primer nucleotide plus the next nucleotide in sequence as determined in step (e), and
 - g) repeating steps (e) and (f), fising the second annealed primer as the starting annealed primer for each repetition, to determine the sequence of the polynucleotide of interest
- 7. The method of any one of Claims 1 to 6, wherein the single base extension reaction comprises subjecting the annealed primers to a reaction mixture comprising a polymerase and nucleotides corresponding to each of the four bases.
- 8. The method of any one of Claims 5 to 7, wherein the nucleotides corresponding to each of the four bases are mutually distinguishable.
- 9. The method of Claim 8, wherein three of the four nucleotides are differently labelled.
- 10. The method of Claim 9, wherein the three differently labelled nucleotides are fluorescently labelled.

- 11. The method of any one of Claims 1 to 10, further comprising analyzing the sequence of the complementary polynucleotide of interest.
- 12. The method of any one of Claims 1 to 11, wherein the terminating nucleotides are dideoxynucleotides.
- 13. The method of any one of Claims 1 to 12, wherein the length N of the oligonucleotide primers is between 7 and 30 inclusive.
- 14. The method of any one of Claims 1 to 13, wherein the length N of the oligonucleotide primers is between 20 and 24 inclusive.
- 15. The method of any one of Claims 1, 2, 13 or 14, wherein the oligonucleotide primers comprise oligonucleotide primers of different lengths.
- 16. The method of any one of Claims 1 to 15, wherein observing the identity and location of a terminating nucleotide comprises the use of a charge coupled device or a photomultiplier tube.
- 17. The method of any one of Claims 3 to 14 or 16, wherein the terminating nucleotides are removed from the annualed primers after completed analysis to prepare the solid support for reuse.
- 18. The method of any one of Claims 1 to 17, wherein the terminating nucleotides are dinucleotides.
- 19. An apparatus for analyzing the sequence of a polynucleotide of interest, comprising a solid support having attached thereon at defined locations an array of oligonucleotide primers having known sequences.
- 20. The apparatus of Claim 19, wherein the oligonucleotide primers are attached to the solid support by a specific binding pair.
- 21. The apparatus of Claim 20, wherein the specific binding pair is biotin and a molecule selected from the group consisting of: avidin and strepavidin.

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